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Challenge of bacteriophage application to improve food safety and its administration into the human gut: an article review

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Abstract

Ensuring microbial food safety has always been a challenge at every stages along the food chain. Meanwhile, healthier community lifestyle demands natural antimicrobial agents to alleviate the increasing use of chemical preservatives to address microbial contamination. Antimicrobial resistance issue has also elevated the effort to search for an alternative way to antibiotics. Bacteriophage (phage) is currently being assessed for its potency as a biocontrol agent to enhance food safety and as a tool for therapeutic purposes. Prior to phage application, safety assessment must be conducted in which includes several considerations: from the discovery, toxicological aspects to the impact of phage ingestion on the gut microbiota. The gut microbiota which consist of variety of microorganisms inside the human gastrointestinal tract, cohabitate to each other. Phage is naturally present as one of microorganisms in the human gut and dynamically interacted with other microbial communities. Phage application to foods and food-contact surfaces may leave a residue and cause the phages to be ingested, which in result may alter the gut microbiota composition. Many findings have examined the relationship between gut microbiota and human health, and so is the factors affecting their modulation. This review aimed to discuss several points of view from published research papers related to the challenge of phage to improve food safety and its administration into the human gut.

Keywords: bacteriophage, biocontrol, food safety, gut microbiota, therapy

Introduction

Pathogenic bacteria, which can cause infectious diseases to human is getting more dangerous. Since the bacteria are dynamically evolving from time to time, and in some cases triggered by the inappropriate use of antibiotics, their ability to survive antimicrobial treatment has become more powerful and adaptive. The high rate of resistance development which is not followed by the discovery of new antibiotics can lead to a serious threat. This event, which is later progressing into multidrug-resistance has become major concern for public health worldwide. In 2019, the Centers for Disease Control and Prevention (CDC) reported that more than 2.8 million people suffered from antibiotic resistance infection and the number of deaths reached more than 35.000 people each year in the United States (CDC 2019).

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Meanwhile, unhygienic condition along the food chain increases the risk of pathogen exposure to the foodstuffs and food-contact surfaces. Treatments with antimicrobial agents may not be effective due to the resistance and biofilm formation (Sillankorva *et al.* 2012; Lewis 2008). Hence, pathogenic bacteria that contaminates the foodstuff will remain viable and can cause foodborne infection when it is ingested at a certain amount. In the United States, the Centers for Disease Control and Prevention (CDC) estimated that there are 48 million cases of foodborne illnesses, of which there are 128.000 hospitalized cases and 3.000 death cases each year. CDC also reported that the major groups causing the illnesses consist of 31 known pathogens and unspecified agents that are transmitted through foods, in which 21 pathogenic bacteria are listed (CDC 2011a; CDC 2011b; Scallan *et al.* 2011). Therefore, in order to reduce foodborne infection and to combat antibiotic resistant bacteria, development of an alternative way to the antibiotics must be encouraged, one of which is by utilizing bacteriophage.

Bacteriophage discovery and therapy

Bacteriophage (or phage) was independently discovered in 1915 by Frederick Twort and later was identified as a virus that can attack bacteria in 1917 by Felix de'Herelle (McKinstry and Edgar 2005). Phages are the most abundant biological entity on Earth, with estimated concentration at 10^{31} (Suttle 2005; Whitman *et al.* 1998). They can be found everywhere in any ecosystems, from the human gastrointestinal tract to the oceans (Dion *et al.* 2020). *Caudovirales* remains as the largest order of bacteriophages. The International Committee on Taxonomy of Viruses (ICTV) reported that this order consists of 9 families, 44 subfamilies, 671 genera, and 1967 species. In 2019, ICTV established new list of *Caudovirales* families, they are: *Myoviridae, Podoviridae, Siphoviridae, Ackermannviridae, Herelleviridae, Autographviridae, Chaseviridae, Demerecviridae,* and *Drexlerviridae* (ICTV 2019). Several published works had classified phages based on the genome type and morphology, which are detailed in Table 1.

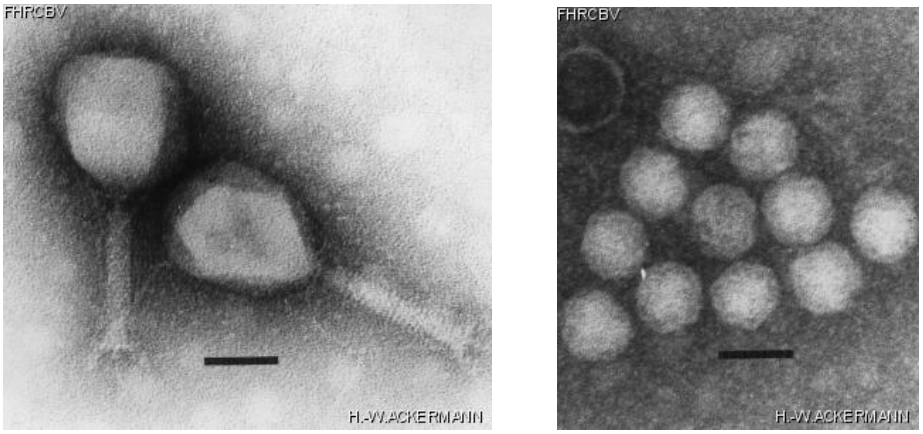

Figure 1. *Escherichia* phage T4 (left) and *Pseudomonas* phage ϕ8 (right). The black bar represents 50 nm.
(Courtesy: the late Prof. Dr. Hans-Wolfgang Ackermann of Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, Canada. Available from: www.phage.ulaval.ca)

Table 1. Classification of bacteriophages*(Adriaenssens *et al.* 2020; Barylski *et al.* 2020a; Barylski *et al.* 2020b; Dion *et al.* 2020; ICTV 2019; Nelson 2014; Ackermann 2011)

ICTV Order	ICTV Family	Genome type	Morphology	Examples
Caudovirales	*Myoviridae*	dsDNA (linear)	Icosahedral head; long, contractile tail	Enterobacteria phage T4, *Campylobacter* phage CP220, *Salmonella* phage FelixO1, *Vibrio* phage KVP40
Caudovirales	*Podoviridae*	dsDNA (linear)	Icosahedral head; short tail	Enterobacteria phage T7, *Streptococcus* phage Cp1, *Bacillus* phage ϕ29
Caudovirales	*Siphoviridae*	dsDNA (linear)	Icosahedral head; long, non-contractile tail	Enterobacteria phage λ, *Mycobacterium* phage Brujita, *Staphylococcus* phage 77
Caudovirales	*Ackermannviridae*	dsDNA	Icosahedral head; long,contractile tail (with tail spikes at the base of the tail)	*Shigella* phage AG3, *Salmonella* phage 38, *Klebsiella* phage 0507KN21, *Serratia* phage MAM1
Caudovirales	*Herelleviridae*	dsDNA (linear)	Icosahedral head; long, contractile tail	*Bacillus* phage SPO1, *Staphylococcus* phage K, *Listeria* phage P100,
Vinavirales	*Corticoviridae*	dsDNA (circular)	Complex icosahedral capsid with internal lipid membrane	*Pseudoalteromonas* phage PM2
Mindivirales	*Cystoviridae*	Segmented dsRNA (linear)	Complex icosahedral capsid, lipid envelope	*Pseudomonas* phage ϕ6, *Pseudomonas* phage ϕ8
Tubulavirales	*Inoviridae*	ssDNA (circular)	Filamentous or rods	*Pseudomonas* phage Pf1, *Escherichia* phage M13, *Vibrio* phage CTXϕ
Levivirales	*Leviviridae*	ssRNA (linear)	Icosahedral capsid	Enterobacteria phage Qβ, Enterobacteria phage MS2
Petitvirales	*Microviridae*	ssDNA (circular)	Icosahedral capsid (12 knoblike capsomers)	*Escherichia* phage ϕX174, *Spiroplasma* phage SpV4, *Chlamydia* phage Chp1, *Bdellovibrio* phage MAC1
-	*Plasmaviridae*	dsDNA (circular)	No capsid, lipid envelope	*Acholeplasma* phage L2, Enterobacteria phage MVL2
Kalamavirales	*Tectiviridae*	dsDNA (linear)	Complex icosahedral capsid with internal lipid membrane	*Bacillus* phage AP50, *Pseudomonas* phage PRD1, *Gluconobacter* phage GC1

*) This classification does not include the following families:*Autographviridae* (*Caudovirales*), *Chaseviridae* (*Caudovirales*), *Demerecviridae* (*Caudovirales*), *Drexlerviridae* (*Caudovirales*),and *Plectroviridae* (*Inoviridae*).

Phage is a virus which can specifically kill targeted bacteria. They can infect bacteria in two ways: lysogenic cycle and lytic cycle. Lysogenic cycle occurs when the environment is not supportive, in this way, phage will only integrate their genome into the bacteria without

causing bacterial cell lysis. In contrast, lytic cycle occurs when the environment is supportive, phage insert their genome and self-replicating inside bacterial host cell until the host lysed (Forde & Hill 2018). Unfortunately, the utilization of lysogenic cycle of phage remains under-explored since most of applied research are mainly focused on assessing the use of the lytic phage instead of lysogenic one to control pathogenic bacteria. Due to its specificity and the ability to lyse the host cell, lytic phage is often projected to be utilized as natural weapon against pathogenic bacteria.

Phage therapy has been used in medicine since 1919, yet this approach was once discredited in the Western World due to the discovery of antibiotics (Summers 2012). Following the resistance of bacteria, reappraisal for phage therapy practice comes after several efforts on controlling pathogenic strains showed effective results, such as the use of lytic phage against *Escherichia coli* in mice (O'Flynn *et al.* 2004), chicken (Huff *et al.* 2002), calves (Smith *et al.* 1987), and against *Pseudomonas aeruginosa* in mouse burn wound model (McVay *et al.* 2007). Until recently, scientists have explored various aspects of the lytic phage from the genome to the metabolome level. Results on phage application in food and clinical setting were also established, for example: phage application to reduce *Salmonella enterica* serovar Enteritidis and *Campylobacter jejuni* in carcass surface (Goode *et al.* 2003), *Pseudomonas lactis* in raw cow's milk (Tanaka *et al.* 2018), *Enterobacter sakazakii* in reconstituted infant formula (Kim *et al.* 2007), *Acinetobacter baumannii* and *Klebsiella pneumoniae* in patient with trauma-related tibial infection (combined with antibiotics) (Nir-Paz *et al.* 2019).

Phage therapy offers promising future as an alternative to antibiotics to control bacterial load and combat antimicrobial resistant bacteria in human and the environment. The advantages of phage therapy outweigh those of antibiotics, in these aspects: 1) effective against multidrug-resistant bacteria since phage and antibiotic have different lysis mechanism, 2) substituted microbism does not occur because of phage's high specificity, 3) adaptive to bacterial mutation that leads to phage-resistance, 4) economic cost to develop phage system is relatively cheaper and 5) side effects are uncommon because phages and their derivatives do not affect eukaryotic cells (Matsuzaki *et al.* 2005).

However, as an old-fashioned way of treating bacterial infection, phage therapy still requires further research to establish guidelines or regulations prior to therapeutic use in human, especially regarding the efficacy and safety following phage exposure.

Key pathogens in human infections: antibiotic resistance and the role of food safety

Global surveillance report published by the World Health Organization (WHO) in 2014 presented the data of bacteria which commonly cause infections in different settings: in hospital, in the community, and transmitted through the food chain. On the list there are seven bacteria of international concern, that consist of: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, Nontyphoidal *Salmonella*, *Shigella* sp., and *Neisseria gonorrhoea*. Typical diseases caused by bacteria included in the report are: urinary tract infections, blood stream infections, wound infections, pneumonia, meningitis, otitis, foodborne diarrhoea, bacillary dysentery, and gonorrhoea (WHO 2014). Furthermore, CDC reported 21 known bacterial pathogens which causing foodborne illnesses and are transmitted through food each year in the United States (CDC 2011b; Scallan *et al.* 2011), as detailed in Table 2.

In addition, WHO also released the global priority list of resistant pathogens which carry the urgency for new treatments. The experts divided the priority pathogens into three main categories: critical, high, and medium. Pathogens that belong to the category of: 1) critical priority, include: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae (*Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp.,

Proteus spp., *Providencia* spp., and *Morganella* spp.), 2) high priority, include:*Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter*, *Salmonella* spp., and *Neisseria gonorrhoeae*, and 3) medium priority, include:*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Shigella* spp. In their publication, the WHO expert panels suggested that the discovery of new treatments to these bacteria should not create further resistance to the existing antibiotics (WHO 2013). Furthermore, CDC classified 18 antibiotic resistant bacteria and fungi into 3 categories based on the threats to human health, which are: 1) urgent (Carbapenem-resistant *Acinetobacter*, *Candida auris*, *Clostridioides difficile*, Carbapenem-resistant Enterobacteriaceae, and Drug-resistant *Neisseria gonorrhoeae*), 2) serious (Drug-resistant *Campylobacter*, Drug-resistant *Candida*, ESBL-producing Enterobacteriaceae, Vancomycin-resistant *Enterococci* (VRE), Multidrug-resistant *Pseudomonas aeruginosa,* Drug-resistant nontyphoidal *Salmonella,* Drug-resistant *Salmonella* serotype Typhi, Drug-resistant *Shigella*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Drug-resistant *Streptococcus pneumoniae*, and Drug-resistant Tuberculosis), and 3) concerning (Erythromycin-Resistant Group A *Streptococcus* and Clindamycin-resistant Group B *Streptococcus*) (CDC 2019).

Table 2. Bacterial pathogens causing foodborne illnesses and their percentage of annual foodborne illness episodes in the United States (CDC 2011b; Scallan *et al.* 2011)

Pathogenic bacteria	Foodborne (%)*
Bacillus cereus	100
Brucella spp.	50
Campylobacter spp.	80
Clostridium botulinum	100
Clostridium perfringens	100
STEC O157	68
STEC non–O157	82
ETEC, foodborne	100
Diarrheagenic *E. coli* other than STEC and ETEC	30
Listeria monocytogenes	99
Mycobacterium bovis	95
Salmonella spp., nontyphoidal	94
S. enterica serotype Typhi	96
Shigella spp	31
Staphylococcus aureus	100
Streptococcus spp. group A	100
Vibrio cholerae, toxigenic	100
V. vulnificus	47
V. parahaemolyticus	86
Vibrio spp., other	57
Yersinia enterocolitica	90

**) percent foodborne among domestically acquired illnesses, based on US population in 2006*

Diseases that are caused by bacterial infection has been taking serious attention since the resistance is progressing quicker than the development of new antibiotics. Bacteria can

originally possess the resistance to certain antibiotic (intrinsic resistance), while other types of bacteria can acquire the resistance genes from the environment (acquired resistance). Resistance can be acquired as a result of mutation or horizontal gene transfer (Verraes *et al.* 2013).

Antimicrobial resistant bacteria can be found in human environments, such as in the soil, plants, water, air, and household area. Food products along the food chain may be vulnerable to be contaminated by the resistant bacteria because of frequent exposure to the unhygienic environment. Plant-based products are susceptible to the contamination because of contaminated soil and irrigation water. One factor that enhances the transfer of resistant gene in agricultural field is the use of manure to soils. Food animals may not only carry the resistant bacteria and the resistant gene, but they can also become the endpoint in the transmission cycle (Thanner *et al.* 2016). The use of antibiotics, transmission from water or fecal materials, and contamination during slaughtering process may render bacterial resistance in animal-based products. Food handler must pay attention to sanitation and hygiene practices during processing to prevent pre-harvest, post-harvest, or cross-contamination as they may occur between raw materials.

Food safety plays an important role. Microbial infection which is carried by elements in the food chain and transmitted to the human body may be the most prominent cause of infectious disease. The gap of knowledge between the transmission of pathogenic bacteria from the environment to the human body needs to be more explored to find the solution. Although the transfer mechanism of resistant genes between bacteria to the environment and human is almost difficult to be demonstrated due to the complex route, limiting bacterial load along the food chain can be the most effective way to prevent the spread of antimicrobial resistance.

Towards implementation of a food safety system, new strategy to control the emerging pathogens must be developed continuously. In this case, phage intervention provides advantages to food safety in ways described by Sillankorva *et al*. (2012), they are: (i) very specific to the targeted host, (ii) ability to replicating and eliminating themselves, (iii) adaptive to changing environments, (iv) low toxicity, (v) relatively low cost and easy to handle, (vi) ability to cope with processing conditions, and (vii) preserving food for a longer time. Phage application along the food chain covers four major functions: as therapy—to reduce bacterial colonization in animals, as processing aid—to decontaminate food contact surfaces and utensils, as antibiotic—to control bacterial load during food processing, and as preservative—to prevent bacterial contamination and growth during storage of final products (Greer 2016). Despite the advantages, there are drawbacks following the use of phage in food setting which has to be further assessed in the safety evaluation.

Nonetheless, to promote surveillance and control of food safety and antimicrobial resistance, the existence of integrated system is essential. WHO pointed out that there are significant gaps in the surveillance and data sharing regarding the impact of antibiotic resistance of foodborne bacteria to the human and animal health (WHO 2014). For example, the concept of "One Health" system that was initiated by WHO, is an effort that brings integrated and multisectoral approach to address public health issue, including antimicrobial resistance and food safety (WHO 2017).

Efficacy and safety concern regarding the application of bacteriophage to human

Food products are essential for human health and well-being. Thus, the development of phage treatment which will be applied in the food chain must consider any potential issues regarding the impact of phage use to the human body. Safety assessment had been conducted by the European Food Safety Authority (EFSA) to evaluate the use of *Listeria* spp. phage,which address the safety of phage application from the toxicological aspects, the

efficacy, the risk of resistance to biocides, bacteriophages, or antimicrobials after phage exposure, and the risk of phage-spreading in the environment (EFSA 2016). Unfortunately, there was no detailed assessment in the potency of phage in modulating gut microbiota in their report yet.

Toxicological safety evaluation evaluates the reaction of chemical substances following phage treatments. EFSA underlined that phage formulation must not present any toxicological problems to human (EFSA 2016). Phage consists of DNA or RNA which was enveloped by protein capsid and can be degraded into amino acids and nucleic acids. The degradation of phage components must not cause any allergic or toxic reactions to human. Furthermore, the administration of phage into the human gut will reduce the concentration of phage due to the low pH of the gastric juice and degradation of protein capsid by digestive enzymes (Dąbrowska 2019). Phage preparation and formulation is important; purified phage in proper dilution will contain less protein elements, so that the safety level is increased (Marza *et al.* 2006).

In regard to the efficacy, phage application must consider the possibility of bacterial resistance toward phages. Citorik *et al.* (2014) reviewed that bacterial resistance can occur as a result of innate mechanism, as in restriction-modification system, and adaptive mechanism, as in clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated (Cas) systems (Citorik *et al.* 2014; Barrangou *et al.* 2007; Tock & Dryden 2005). The efficacy of phage utilization depends on the specificity of phage, the potency of emerging resistant mutant, and preservation ability to longer shelf life. Phage intervention confers therapeutic efficacy when phage is selected from these criteria: safe, lytic, polyvalent, stable,self-replicating, and work effectively when combined in a cocktail (Loc-Carrillo & Abedon 2011, El Haddad *et al.* 2016).

The risk of resistance to biocides must be evaluated particularly for the group of compounds that is frequently used, such as quaternary ammonium compounds (QACs) which often found to be used in food industry and hospitals. The risk of resistance to phage following exposure may occur because of the absence or changes of the phage receptor, the presence of CRISPR-Cas systems which is related to bacterial resistance against phage and plasmids, and unsuccessful infection. EFSA provided several evidences on the occurrence of bacterial resistance to phage after long exposure of *L. monocytogenes* to phage therapy (EFSA 2016). The risk of resistance to therapeutic antibiotics may vary between strains. In *L. monocytogenes*, the resistance to therapeutic antibiotic arises after the assembly of self-transferable plasmids, mobilizable plasmids and conjugative transposons (Charpentier & Courvalin 1999). The resistant genes in phage can be detected through molecular analysis by using sequencing technique.

Safety assessment of phage application must include environmental aspects. Evaluation of waste components which are resulted from the application of phage should devise the possible released dosage and information on the exposed site. The inherent simplicity of phage metabolism supports an idea that phage may be lasted in the environment for long period of time, but due to phage susceptibility to temperature change, UV light, and microbial enzymes produced in the environment, phage is likely to undergo inactivation and break down (EFSA 2016). Still, the possibility of phage reactivation that may initiate the event of bacterial resistance upon its release from phage preparation should be taken into consideration.

Route of administration and modulation of gut microbiota

Delivering phage into the human body can be carried out by using phage therapy, phage-treated foods, food products, and other environment-exposed components. Phage therapy can be administered through various routes: oral, intravenous, intra-duodenal,

intramuscular, and rectal (Abedon *et al.* 2011). Pharmacological factors, both pharmacokinetics and pharmacodynamics, decide the triumph of phage therapy. Pharmacokinetics regard the half-life of phage, while pharmacodynamics regards the virulence of phage (Forde & Hill 2018). Basically, there is a different concept of pharmacokinetics between chemical drugs and phage therapy. Phage pharmacokinetics combine the theory of antibacterial pharmacokinetics with consideration of phage behavior (Dąbrowska 2019). Therefore, all the aspects which are related to ecological perspective of phage must be regarded.

The oral route is thought to be the most convenient way for the delivery of phage therapy since oral administration of therapeutic agents is generally fitting in with patients. Phage that is intended for treating bacterial infection in the gastrointestinal tract as well as other human organs is often used in this route of administration. One important factor affecting the success of oral phage delivery is the ability of phage to survive gut transit. The success of phage in gut transit is commonly examined by detecting phage recovery in feces after oral application. Literature study of experimental phage showed that they can pass through the human gut, and their recovery depends verily on the amount of ingested dose (Dąbrowska 2019). An example of an established success of oral phage therapy was provided by Leszczyński *et al.* (2006) in their study for eradicating MRSA colonization in the gastrointestinal and urinary tract.

Gut microbiota profile has been well-understood to have the correlation with human health. Hence, phage, as a natural member of gastrointestinal tract, also takes the role in affecting human health. Metagenomic approach was used to understand the mechanism of gut microbiota modulation triggered by phage. Temperate phage mostly inhabits healthy gut, while virulent phage indicates gut dysbiosis (Norman *et al.* 2015).

Ingestion of certain therapeutic agents will alter gut microbiota composition and bring impact to the patient's health. Therapy by using fecal microbiota transplantation (FMT) was previously showed successful result to relieve the infection of *Clostridium difficile* (Gupta *et al.* 2016). It was believed that FMT contributes to the transfer of lytic phages, either carried along with their host or conquered a new host within the patient's gut microbiota (Draper *et al.* 2018). Report by Zuo *et al.* (2017) stated that theFMT therapy alter the enteric viromes composition (including the Caudovirales or phages) after weeks of treatment in patients with *Clostridium difficile* infection (CDI) (Zuo *et al.* 2017). However, the U.S. Food and Drug Administration (FDA) in 2019 warns the practice of FMT, as it possesses serious adverse effect to human with the risk of the transfer of antibiotic resistant bacteria (FDA 2019). Taking the warning into account, the research on FMT practice should be more reviewed and explored to answer the safety concern.

Phage therapy can affect the physiological condition due to the increasing or decreasing amount of phage and bacteria (the changing ratio). An illustration of this impact is shown in a study comparing the phage composition between the healthy adults and adults suffer from traveler's diarrhea. In healthy adults, there were only low concentration of phage with the type of lambda-like phage, while in the patients, the phage concentration were higher with the type of T4-like phage (Chibani-chennoufi *et al.* 2004).

Regulatory hurdles

Phage therapy research and development has been extensively studied to the level of clinical trials. Regardless of the exploratory study, there is still no official published guidelines in the Europe and United States that cover the development and clinical study of phage application. In contrast, there are commercially available phage products which have been approved for use in food settings. In the agricultural field, there is AgriPhage (OmniLytics™) which is intended to treat bacterial spot disease in crops. In food industry,

Listex™ P100 (EBI Food Safety) is used as an antimicrobial agent to control the growth of *L. monocytogenes* in cheese, meat, and poultry products which has been granted "Generally Regarded As Safe (GRAS)" status from the US FDA (EFSA 2016). To obtain the approval, dossiers which include scientific evidence that address the efficacy and safety of phage product, including the pharmaceutical, pre-clinical, and clinical aspects, must be submitted to be reviewed by the experts.

In the Europe, phage therapy is regarded under the European regulatory framework on biological medicinal product in Directive 2001/83/EC. Due to the dynamic characteristic of phage, the application dossier must include detailed information on phage characterization which stated critical parameters to the quality and safety, the specifications and acceptance criteria, and the flow manufacturing process (Pelfrene *et al.* 2016). Until recently, there is no regulatory scheme in which focuses to evaluate the ecological aspect of phage and health care support in providing phage therapy. In the future, it is expected that the regulatory development in the United States will grow rapidly since the US FDA currently permitted the use of phage therapy to support the study at the Center for Innovative Phage Application and Therapeutics (IPATH) via the Emergency Investigational New Drug scheme (Furfaro *et al.* 2018).

In conclusion, phage therapy offers promising approach to fight against the spread and infection of resistant pathogenic bacteria in the food chain. There were many scientific reports supporting the application of phage therapy in food and clinical settings, but the data on the mechanism after phage exposure to modulate gut microbiota is still limited. Further data is still required since phage administration to the human body must be scientifically proven safe and effective. Scientific evidence regarding the use of phage is also needed as basic framework for drafting regulatory products.

Conflict of interest

The author declared that no possible conflict of interest from this manuscript.

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